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COMPARATIVE ULTRASTRUCTURAL STUDY ON THE NEUROMUSCULAR JUNCTION IN THE MUSCLES OF THE ALIMENTARY CANAL OF *LOCUSTA MIGRATORIA* AND *HELIX POMATIA*

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Abstract

The ultrastructure of the neuromuscular junctions in the gastrointestinal system of the migratory locust *(Locusta migratoria migratorioides)* and the snail *(Helix pomatia)* was studied comparatively.

Large amounts of synaptic and non-synaptic nerve terminals were found in the insect gut muscle. In the snail intestinal wall on the other hand, where the axolemma tightly fitted to the sarcolemma, the nerve terminals did not form neuromuscular synapses.

In both species, high numbers of non-synaptic terminals were found in which neurosecretory granules of varying electron density were present. Authors regard these terminals as peptidergic ones.

In the authors' opinion, the dense-cored axon terminals occurring in lower numbers are aminergic terminals.

Neurons were not detected in the insect, gut however, neurons containing peptidergic as well as those containing aminergic granules were found in the snail gut.

Key words: Neuromuscular junction in the gut of locust and snail

Introduction

Studies on the morphological characteristics, innervations and neuromuscular junctions of the muscular system of the gastrointestinal tract are mainly limited to the mammalian (rat, guinea-pig, rabbit, etc.), including human intestinal canal (GABELLA, 1979; GORDON—WEEKS, 1981, 1982; LLEWELLYN—SMITH et al., 1981; FAUSSONE—PELLEGRINI, 1983). Only sporadic data have been reported for other vertebrates, e. g. SALIMOVA and FEHÉR (1982), using microspectrofluorimetric and electron-microscopic methods, studied the innervation of the alimentary tract in chondrostean fish.

As to the neuromuscular junction of invertebrates, mostly Arthropods, among them a few insect species, viz. *Periplaneta americana, Locusta migratoria, Schistocerca gregaria,* etc., have been studied. ANDERSON et al., (1977, 1978) described neuromuscular synapses in the outer layer of the three-layered striated gut muscle of *Schistocerca gregaria.* BENEDECZKY and MILLER (1983) carried out studies on the aminergic and peptidergic innervation of the hindgut of *Periplaneta americana* and *Locusta migratoria.* ECKERT et al., (1981), applying an immunocytochemical method, demonstrated a peptidergic transmitter, the proctolin (BROWN, 1975), in the hindgut of the cockroach.

There are few literary data concerning other invertebrate phyla in respect to the neuromuscular junction (TÁNCZOS and TÁNCZOS, 1979; ÁBRAHÁM, 1983). The *Mol-*

lusca phylum, for example, is worthy of note, since the intestinal canal of the included species, similarly to the vertebrate intestinal canal, contains smooth muscle, the muscular system of the alimentary canal of Arthropods, on the other hand, is striated as insect muscles in general.

Since the gut muscle of different structure serves the same physiological functions in both phyla, it seemed justified to compare the gut muscle of a representative of each phylum (*Arthropoda* and *Mollusca*), with particular reference to the ultrastructure of the neuromuscular junctions.

Materials and methods

Mature male and female individuals of *Locusta migratioria migratorioides (Arthropoda: Insecta)* and *Helix pomatia (Mollusca: Gastropoda)* were used. The migratory locusts were obtained from the breed of the Biological Research Institute of the Hungarian Academy of Sciences, Tihany. The snails were collected in humid meadows, near Szeged. The animals were dissected and 1 mm^3 pieces cut from various parts of the alimentary tract were fixed in cold state in 2.5% glutaraldehyde (diluted with cacodylate buffer) for 2h at pH 7.3. Following this, the tissue blocks were washed in cacodylate buffer containing 7.5% sucrose. After washing, the material was postfixed in 2% osmium tetroxide, buffered with s-collidin (pH: 7.3) at +4 °C for 2h, then dehydrated in an ascending ethanol series. The tissue blocks were contrasted for 1h in 75% ethanol with saturated uranyl acetate. The blocks were embedded in Spurr embedding matter, and sections were prepared. The sections were contrasted with lead citrate, and photographs were taken in a TESLA BS 500 electron microscope.

Results

Both the striated gut muscle of *Locusta migratoria* and the gut smooth muscle of *Helix pomatia* are rich in nerves (Figs 1, 2). The sarcoplasm on the gut muscle fibres of *Locusta migratoria* (Fig. 1) forms processes of regular arrangement. The interstitial space surrounding the muscle fibres contains large amounts of collagen fibres; tracheoblasts and smaller tracheoles occur at places. Nerve fibres and terminals are also situated there. The nerve fibres are surrounded by glial processes, the axon terminals are often surrounded by the processes of the muscle fibres. The smooth muscle fibres are of varying courses in the gut muscle layer of *Helix pomatia* (Fig. 2). Omega-profiles and vesicles referring to exocytosis are rather frequent at the rims of the muscle fibres. The nerve bundles and nerve fibres run parallel with, or perpendicularly to the muscle fibres in the interstitial space, which is rich in collagen fibres. The membranes of the terminals are in tight connection with the sarcolemma at places, and here too, the nerve fibres not associated with muscle fibres are covered by processes of glial cells.

On the basis of the fine-structural characteristics of the granules and vesicles found in the nerve fibres and terminals, it can be stated that several kinds of fibre types and axon terminals play a role in the innervation of the muscles in both species.

Synaptic (Figs 3—5) and non-synaptic (Figs 6—7) nerve terminals can be distinguished in *Locusta migratoria*. The axon terminals forming synapsis always contain clear vesicles around 50 nm in diameter. The majority of the vesicle population of these terminals are formed by clear vesicles (Figs 3, 5); in other presynaptic elements, (Fig. 4), besides the clear vesicles, there are larger (diameters of 130 nm) granules of variable density (Fig. 4) in great numbers. Neuromuscular junction may

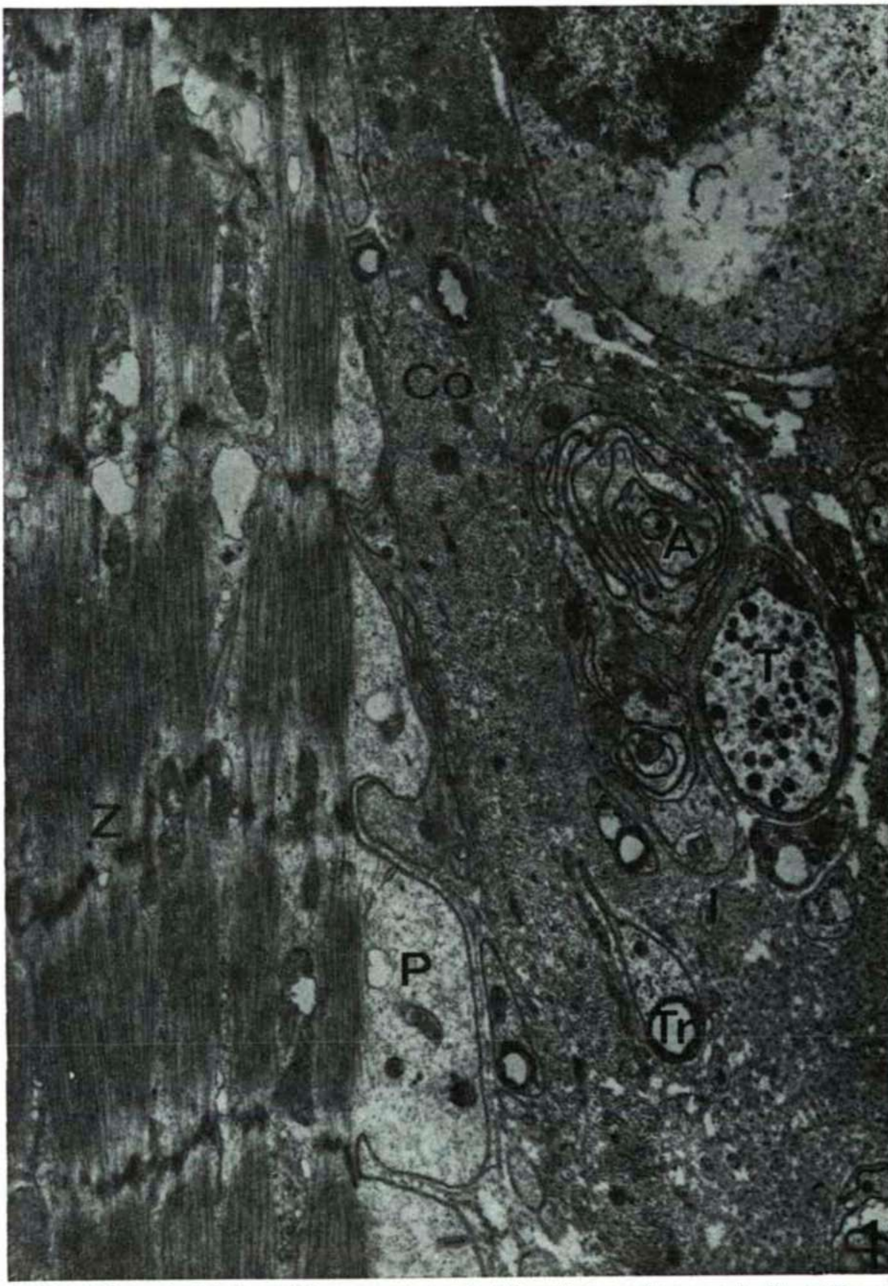

Fig. 1. Detail from the hindgut of *Locusta migratoria*. The striation of the muscle fibres (Z) and the protrusion (P) of the sarcoplasm can well be seen. Note the many collagen fibres (Co), various types of axons (A), and axon terminals (T) in the wide interstitium (I). Tr = tracheola

x 10,000

Fig. 2. Longitudinal section from the smooth muscle layer of snail gut. Besides the nerve bundles (NB) running parallel with the smooth muscle fibres (Mf), nerve fibres (N) running perpendicularly to the muscles are seen. Latter form tight morphological connection (arrow) with the muscle fibres at places. Co=collagen x 9900

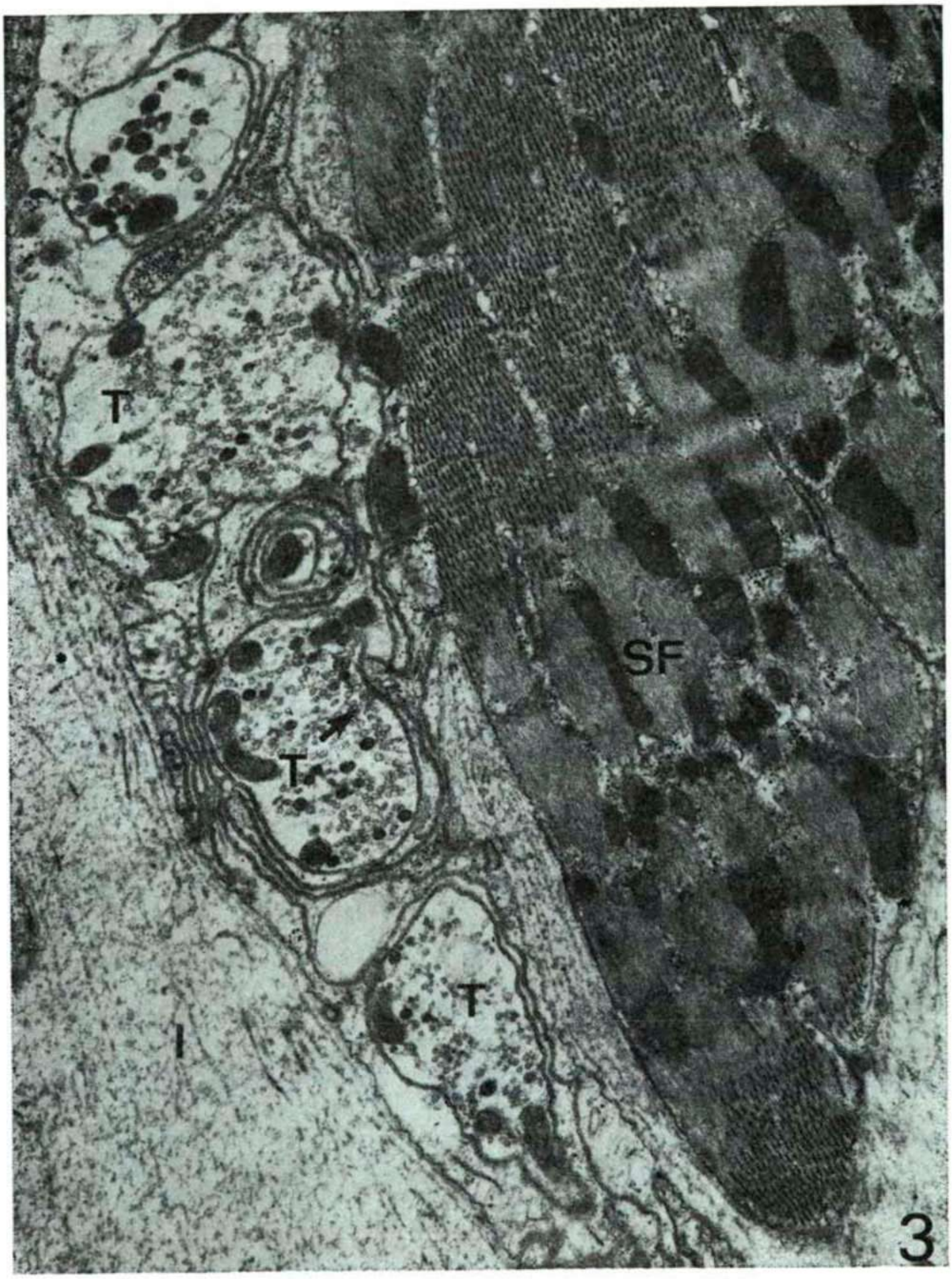

Fig. 3. The axon terminals (T) in the neighbourhood of striated muscle fibres (SF) of *Locusta migratoria* hindgut mostly contain clear vesicles and develop neuromuscular synapsis (arrow) with muscle processes. I = interstitium x 16,000

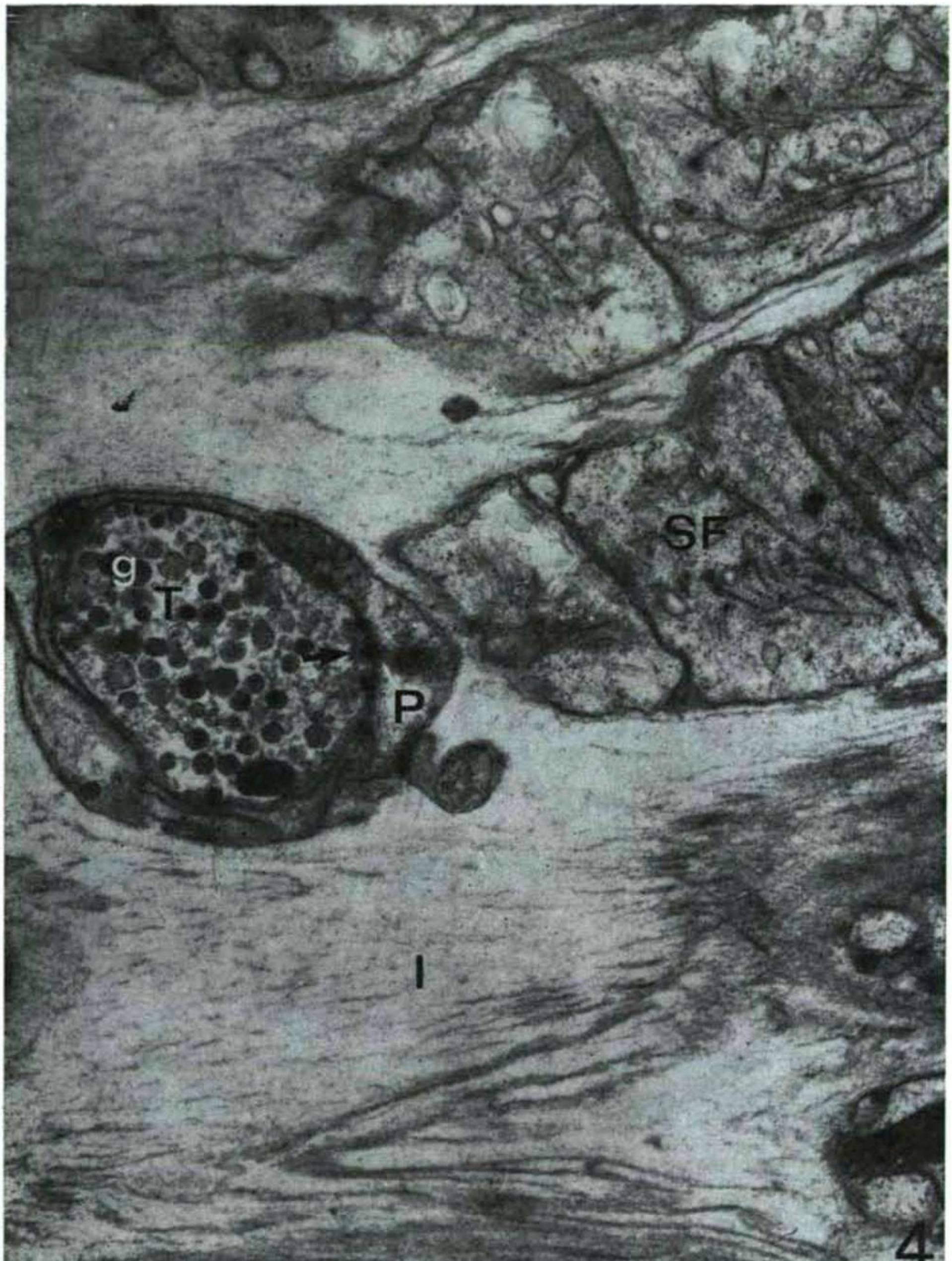

Fig. 4. Synapsis (arrow) between the process (P) of a striated muscle (SF) of *Locusta migratoria* hindgut and a terminal (T) which contains, apart from the clear vesicles, a great number of large, moderately electron dense granules (g). I = interstitium x 16,000

Fig. 5. High magnification of a neuromuscular junction in the gut musculature of *Locusta migratoria*. SF = striated muscle fibre; T = nerve terminal; C = accumulation of vesicles along the presynaptic membrane-thickening. x 70,000

develop between a process of the muscle and the axon terminal (Figs 3, 4), or the axon terminal adjacent to the sarcolemma of the muscle fibre forms a synapsis (Fig. 5). The other, non-synaptic, group of the nerve fibres contains only few clear vesicles, if at all, the terminals are filled by large (150 nm), highly electron dense granules (Figs 6, 7,). These also lie in the direct neighbourhood of the muscle fibres, often adjacent to the sarcolemma, however, synaptic relation cannot be observed in the case of this terminal type.

Several kinds of nerve fibres can be found among the gut muscle fibres of *Helix pomatia,* too. Fibres and terminals containing large (200 nm in diameter), highly electron dense granules occur here, too (Fig. 2, 8). The electron dense granules are mostly of ellipsoidal shape, in contrast to the spheroid form of the granules in insects. The electron density of the granules is also more variable than in the non-synaptic terminals of *Locusta migratoria:* moderately and highly electron dense granules are regularly found within the same terminal (Figs 2, 8). Two further types of nerve fibres occur in *Helix pomatia,* viz, those having so-called large (100 nm in diameter) dense-core vesicles (Fig. 8), and terminals containing moderately electron dense granules with large diameters (250 nm) and granular matrix (Figs 2, 9). The latter type occurs less frequently than the former. The latter two fibre types can be found, in the insect gut rarely. All three listed nerve fibre types may establish tight morphological contact with the muscle fibres (Figs 2, 8, 9), however, membrane specialization characteristic of the neuromuscular synapsis and accumulation of clear vesicles were not observed at all in the snail gut.

Not only nerve fibres, but even neurons were detected among the muscle fibres of *Helix pomatia* (Fig. 10). The perikaryon of the neurons is almost full of electron dense, variously shaped, mostly ovoid, granules. The cell nucleus is rich in chromatin, and besides the granules, the most characteristic organelles in the perikaryon are tubules of the rough-surfaced endoplasmic reticulum. Apart from the relatively small mitochondria, myeline figures and vacuoles occur in the cytoplasm of the neurons.

Discussion

During our comparative ultrastructural studies on the neuromuscular junctions of the *Locusta migratiora* and *Helix pomatia* guts, two basic differences were observed: First, in the snail gut we have not found neuromuscular junction referring to chemical impulse transmission in the classical sense.

Second, the uni-, bi- and multipolar neurons being present in significant amounts in the snail gut were absent in the insect gut.

It is difficult to give an unambiguous explanation for the two morphological differences. The fact that the gut wall of the snail is formed by smooth muscles is phylogenically a morphological and functional feature characteristic of vertebrates. The organization of the rich innervation observed by light microscopy in the snail gut muscle resembles the AUERBACH's plexus of vertebrates in many respects. In the AUERBACH's plexus, as well as in the snail gut muscle, the axon terminals do not form synaptic connection with the sarcolemma. In the insect gut muscle, on the other, hand, the many synaptic connections are noteworthy. These are not completely identical in shape with the skeletal muscle's neuromuscular motor end plates of vertebrates, but the pre- and postsynaptic thickening can well be identified here

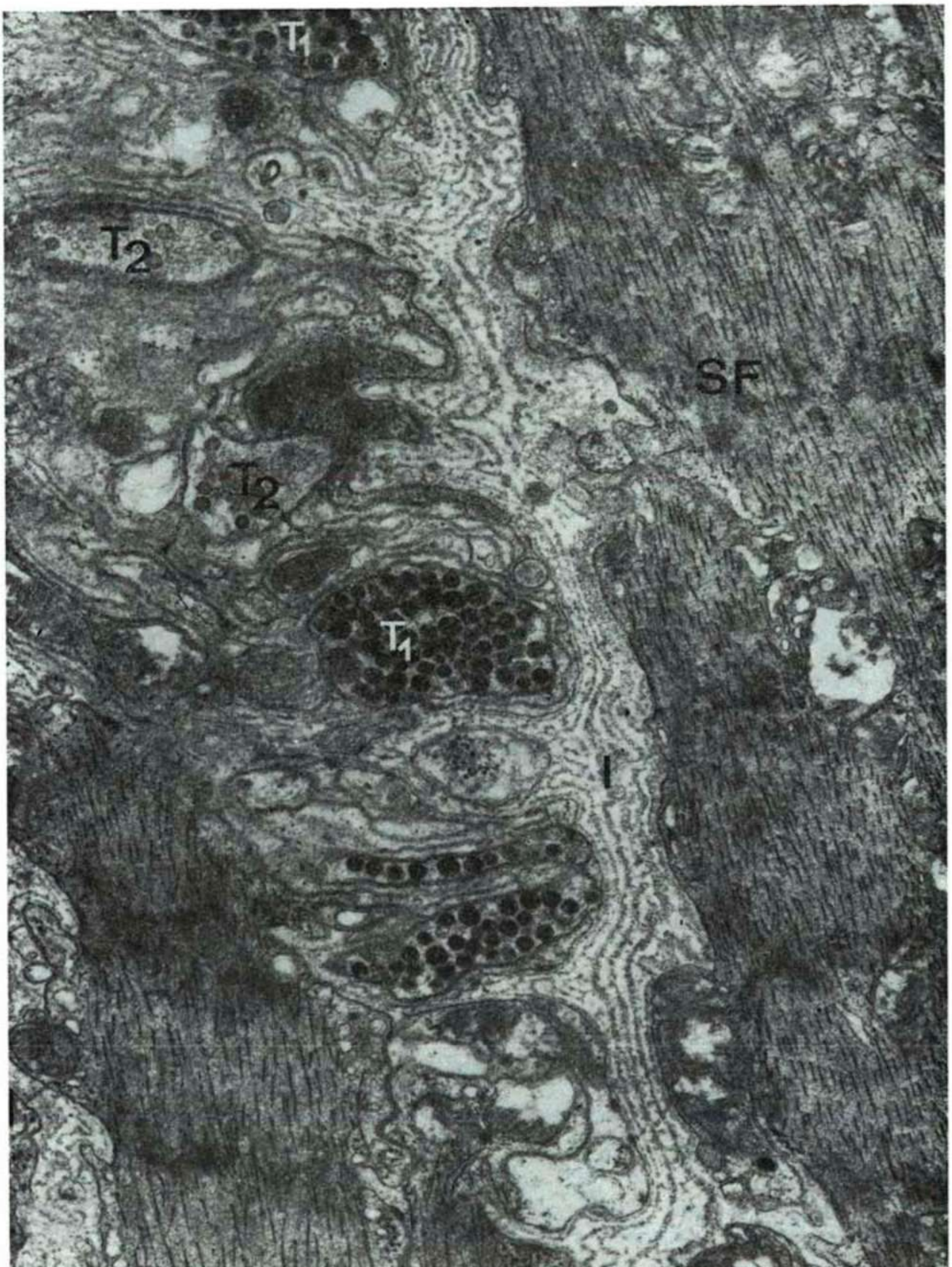

Fig. 6. Neurosecretory nerve fibres (T_1) among striated muscle fibres (SF) of *Locusta migratoria* gut. Note the synaptic terminals (T_2) with clear vesicles. I = interstitium x 16,000

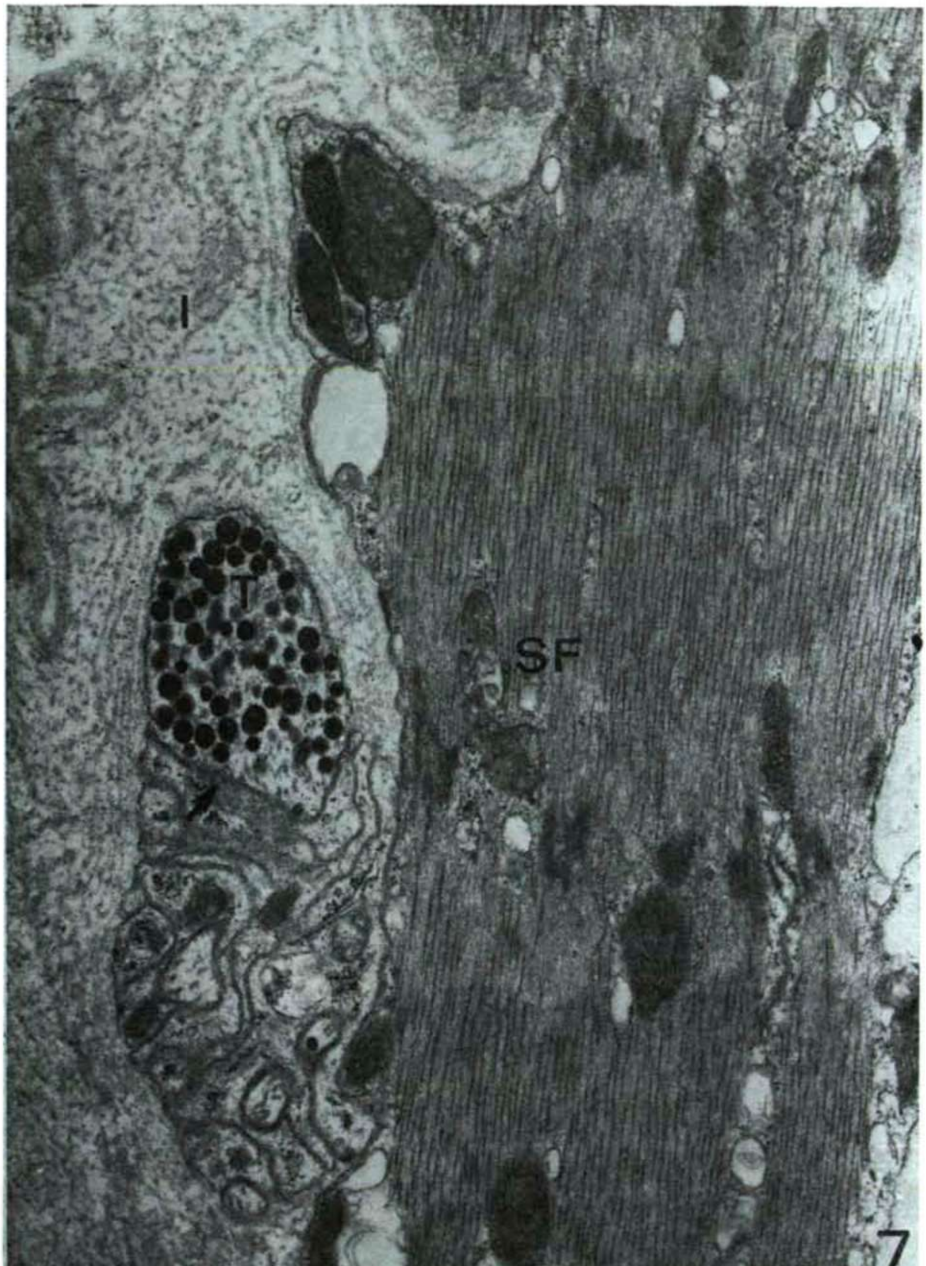

Fig. 7. Tight connection (arrow) between an axon terminal (T) filled with neurosecretory granules and a striated muscle process (SF) in the hindgut of *Locusta migratoria*. The membrane of the axon terminal is not surrounded by cytoplasmic process of glia cell, therefore, this is in direct contact with the interstitium (I). x 16,000

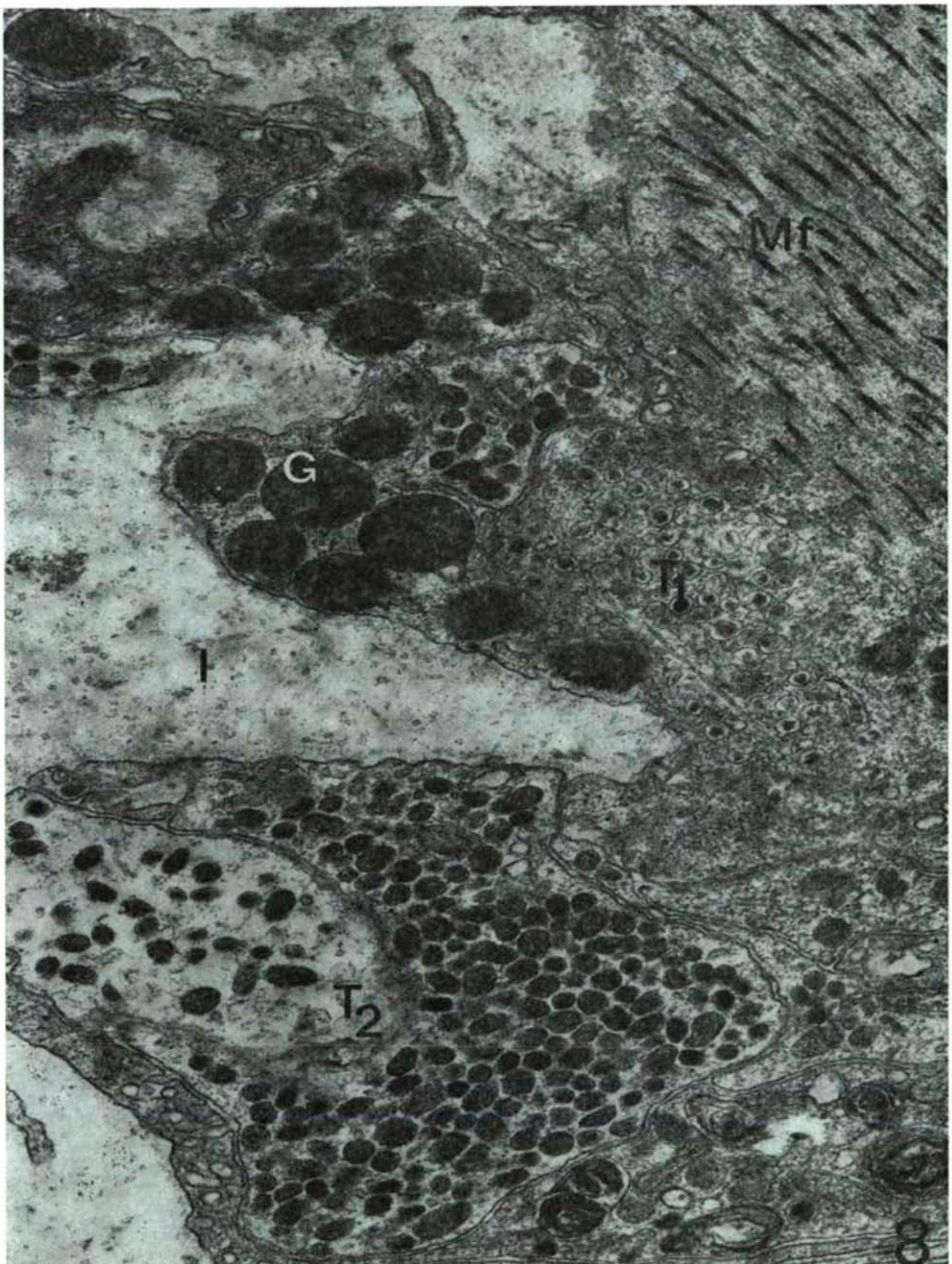

Fig. 8. Smooth muscle fibre (Mf) of snail gut being in tight morphological contact with nerve fibres containing dense-core vesicles (T_1) and those containing neurosecretory granules (T_2). G = glia cell process with granules. I = interstitium x 12,000

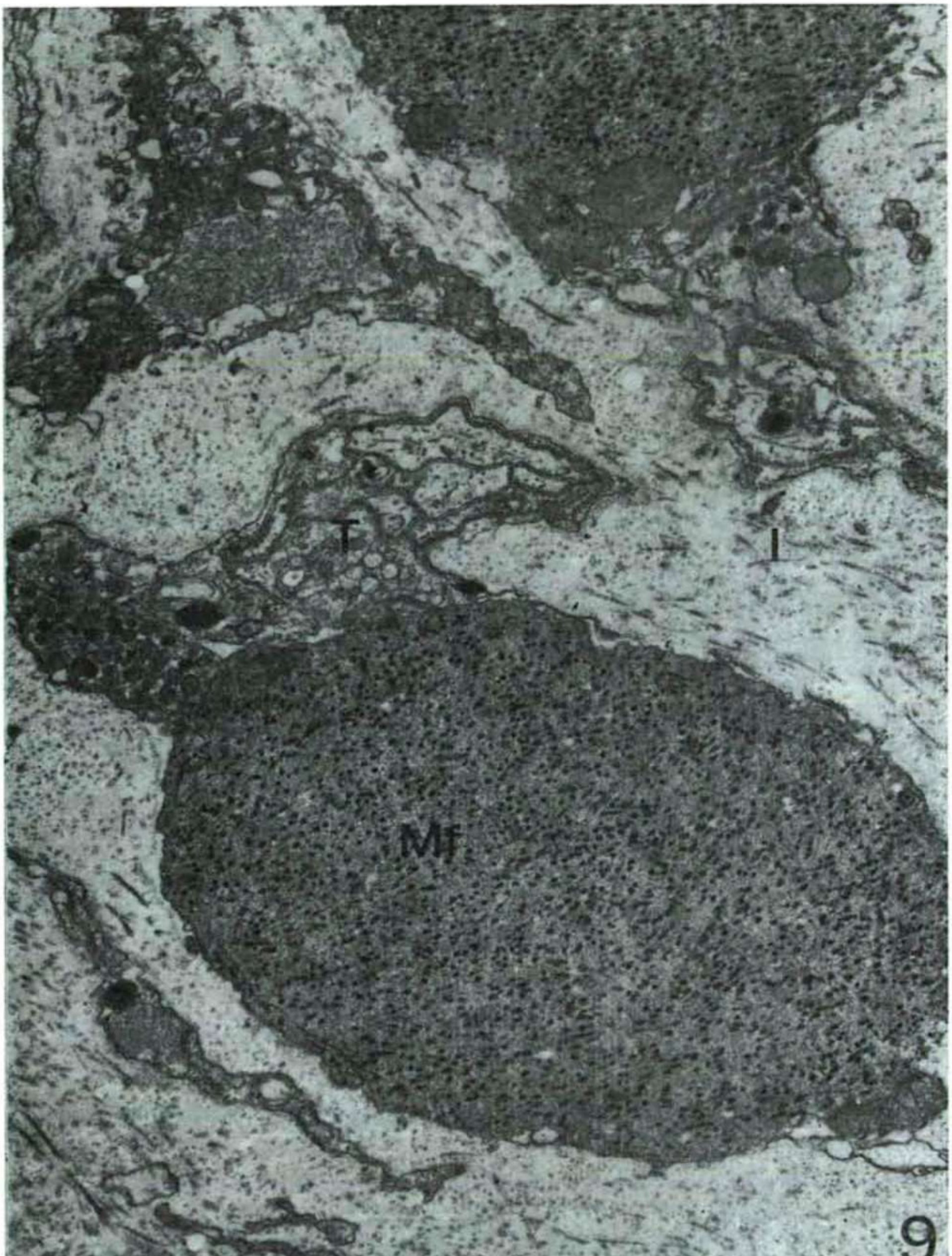

Fig. 9. Cross section of smooth muscle fibre (Mf). In its neighbourhood, note the nerve fibre (T) containing moderately electron dense granules with granular matrix. I=interstitium

x 18,000

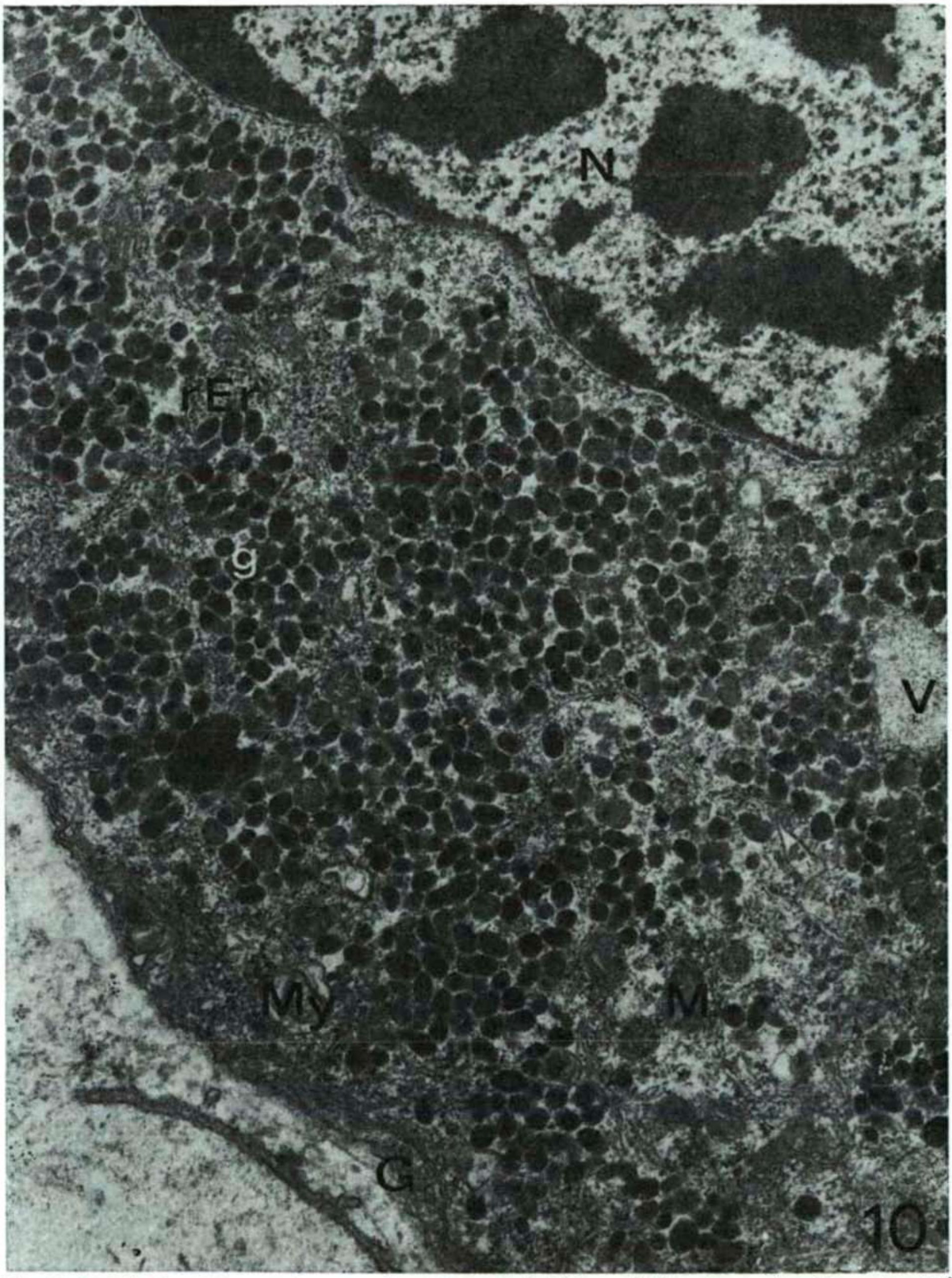

Fig. 10. Detail of an intramural neuron from the gut of edible snail. The chromatin-rich nucleus (N) is surrounded by cytoplasm packed with neurosecretory granules (g), large amounts of rough-surfaced endoplasmic reticulum (rEr) and mitochondria (M). The cell is covered by glial process (G). My = myeline figure, V = vacuole x 23,000

too, and even the accumulations of vesicles are detectable along the presynaptic membrane thickening (BENEDECZKY and MILLER, 1983). All these characteristic structural features refer to the fact that the functional characteristics of the striated muscular system of the insects gut differs in many respects (physiological behaviour, pharmacological sensitivity) from the common, classically-regarded physiological characteristics of smooth muscles. Surely, this is related to the characteristic habitude of insects, for example, the intensive mobility (see the long-range migration of *Locusta migratoria*), which has resulted in the development of a specific muscle system.

Regarding the other main characteristic difference, the presence of neurons in the snail and their absence in the insect, it is known that the gut muscles of the insects are supplied with central nerve fibres, viz. with nerves of the stomato-gastric nervous system in the area of the fore- and midgut, and with nerves coming from the ventral ganglions in the area of the hindgut (ANDERSON and COCHRANE, 1978). Neurons in the muscle layer of insect gut have only been reported by REINECKE et al., (1978), who found multinuclear, peripherally located neurosecretory neurons in the hindgut of *Manduca sexta* larvae. Comparing these cells with those described by us in *Helix pomatia,* we found essential differences: the neurosecretory cells in the insect gut are multinuclear with nuclei poor in chromatin, and the plasma is less abundant in neurosecretory granules than the neurosecretory cells of the snail. Investigations with physiological methods should elucidate the function of the neurons in the gut of insects and snail. On the basis of the heterogeneous ultrastructural picture of the neuromuscular junctions observed in the gut muscle of *Locusta migratoria* and *Helix pomatia,* it is presumable that various chemical substances may play a role as transmitter in the innervation of both striated and smooth muscles.

Several authors have attempted to elucidate the transmitters belonging to the various types of vesicles and granules in the AUERBACH's plexus of mammals (KOMURO et al., 1982, GORDON—WEEKS, 1981, 1982). The classification given in the survey of GABELLA (1979), suggesting that the synaptic, fibres containing clear vesicles are cholinergic, whereas those containing dense-core vesicles are aminergic has been queried by many others. GORDON—WEEKS (1982), e. g. demonstrated in experiments applying false transmitter (5-OHDA, 6-OHDA and 5,6-DHT) treatment that not every fibre containing dense-core vesicles can be regarded as noradrenergic, furthermore, that the apparent size and shape and the intactness of the vesicles greatly depend on the fixative. Therefore, the chemical nature of the transmitters cannot be judged merely on the basis of the morphological picture. It is also questionable to what extent the results of experiments carried out on mammals can be applied to invertebrates.

There is no doubt that the fibres containing large, highly electron dense granules — as we have demonstrated in the case of *Helix pomatia* — are the axons of one of the neurosecretory cells. The literature abounds in data suggesting that the large neurosecretory granules contain a neurosecretion of peptide-nature (RAABE, 1982). It is also supported by literary data that the neurosecretory granules leave the terminals through exocytosis (BENEDECZKY and MILLER, 1983) and exert their effect on the functioning of the muscule fibres. In the case of *Locusta migratoria,* it is also very likely that one of these neurohormones is identical with proctolin, a pentapeptide which was isolated from insect gut by BROWN and the occurrence of which in the hindgut of the cockroach was demonstrated by ECKERT et al., (1981), using an immunocytochemical method.

The exact chemical structure of the transmitter substances of the snail neurosecretory fibres is still to be elucidated. Our assumptions, according to which the nerve terminals containing large dense-core vesicles may be aminergic, seem to be supported by biochemical data and our current histochemical studies (NEMCSÓK et al., FEKETE, personal communication).

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